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Title: Effect of non-surgical periodontal therapy in hypertensive patients with assessment of Endocan and TNF-α levels in gingival crevicular fluid (Case control study)

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Effect of non-surgical periodontal therapy in hypertensive patients with assessment of Endocan and TNF-α levels in gingival crevicular fluid

(Case control study)

Protocol Submitted to the Department of Oral Medicine, Periodontology and Oral Diagnosis
Faculty of Dentistry, Ain Shams University
In partial fulfillment of the requirements of Masters' degree in periodontology

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I. Abstract

Statement of problem: There is a strong crossway relationship between periodontitis and hypertension. Hypertension is the most prevalent of cardiovascular diseases and its prevalence is increased by the presence of periodontitis. Periodontal attachment loss is higher in hypertension patients when compared to healthy subjects. Both are examples of non-communicable diseases (NCDs), also known as chronic inflammatory diseases, they tend to be of long duration and are the result of a combination of genetic, physiological and environmental factors. The lack of an appropriate balance between these factors results in cellular changes as well as the levels of cytokines with subsequent changes in clinical manifestations of periodontal diseases. Both periodontitis and hypertension are associated with inflammation and an increase in specific proinflammatory cytokines.

Aim of the study: To investigate the correlation between the clinical parameters and the levels of Endocan and TNF-α in gingival crevicular fluid (GCF) before and after non-surgical periodontal therapy (NSPT) in stage II periodontitis patients with hypertension in comparison to non-hypertensive patients.

Materials and Methods

Clinical assessment of specific clinical parameters: plaque index, gingival index, probing depth, clinical attachment level (CAL) as primary outcome. Biochemical assessment of Endocan and TNF-α levels in GCF by Enzyme Linked Immunosorbent Assay (ELISA) as well as measurement of blood pressure at baseline and after 3 months as secondary outcomes.

II. Background:

Hypertension and periodontitis are part of a group of diseases called non-communicable diseases (NCDs), which are rising in prevalence due to an increasingly aging population. NCDs comprise 71% of global death.

Hypertension is defined as repeated office SBP values ≥ 140 mmHg and/or DBP ≥ 90 mmHg. It is a multifactorial disease, that can be caused by endothelial dysfunction (ED), oxidative stress, and inflammation. Periodontitis is a chronic multifactorial inflammatory condition affecting the tooth periodontium. In periodontitis, chronic inflammation occurs in reaction to untreated bacteria, and this is associated with continuous stimulation of the immune system which subsequently causes damage to the tooth supporting tissues. The damage is manifested clinically with gingival bleeding, foul odor, clinical attachment loss, tooth mobility and may lead eventually to tooth loss.

The interaction between bacteria and host immune responses is the link between periodontitis and hypertension along with a number of chronic systemic diseases, such as diabetes mellitus, other CVDs and neurological diseases such as Alzheimer.

Patients with periodontitis often present with higher arterial BP values and a 30% to 70% higher chance to also present with hypertension, especially when there is active gingival inflammation.

Periodontitis patients express elevated levels of local and systemic inflammatory biomarkers, such as C-reactive protein (CRP), TNF-α, neutrophilic enzymes, WBCs, disparity in T-cell subtypes, neutrophil dysfunction, which are all mechanisms resulting in vascular changes and endothelial dysfunction (ED).

The presence of periodontal pathogens has been linked to hypertension in epidemiological studies. Periodontal pathogens cause prolonged T-cell activation and elicited increased levels of CRP, TNF-α, and IL (interleukin)-1β, resulting in an increased BP.

The first study to suggest a correlation between periodontal pathogens and hypertension was INVEST (infections and vascular disease epidemiology study) in 2012, its results concluded a direct relationship between the levels of subgingival periodontal bacteria and both systolic blood pressure (SBP) and diastolic blood pressure (DBP) as well as hypertension prevalence.

Endothelial Dysfunction (ED) is one of the early signs of hypertension, Endocan is a biomarker of ED, it also plays a role in endothelium-dependent pathological disorders including cardiovascular diseases (CVDs) and periodontitis. This was the trigger to suggest Endocan as a clinical biomarker for hypertension in 2013.

A consensus report in 2020 showed that there is evidence from epidemiological studies that periodontitis patients exhibit significant endothelial dysfunction, measured by flow‐mediated dilation (FMD), arterial stiffness (e.g., pulse wave velocity—PWV) and a significantly greater thickness of the carotid intima media (cIMT) and elevated arterial calcification scores. This explains why both periodontitis and hypertension are correlated by endothelial dysfunction.

Endocan, previously known as Endothelial cell-specific molecule-1 (ESM-1) is one of the molecules secreted by endothelial cells. Endocan is a 50 kDa soluble proteoglycan that regulates many major cellular activities like cell differentiation, cell proliferation, VCAM-1 and ICAM-1 expression, regulation of leukocyte migration, neovascularization, and metastasis of tumors.

Endocan was recognized of being overexpressed in inflammatory conditions, CVDs, sepsis, cancer, and obesity along with increase in the secretion of vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and intercellular adhesion molecule-1 (ICAM-1). Therefore, Endocan has been designated as a proinflammatory mediator that binds to Lymphocyte function-associated antigen-1 (LFA-1) on human leukocytes and regulates LFA-1 interactions with ICAM-1. Previous evidence indicates that Endocan is increased in inflammatory diseases and inflammation has already been shown to have an important contributing part in the pathogenetic changes in both periodontitis and hypertension.

Serum Endocan levels were significantly higher in the HT group ($P < .001$). Also, it correlated positively with carotid intima-media thickness (cIMT), and high-sensitivity C-reactive protein (hsCRP) which are pathological indicators of atherosclerosis and hypertension.

A systematic review conducted from 20 studies in 2023, showed that in hypertensive patients circulating Endocan levels are significantly elevated. Thus, suggesting endocan as an easy-to-use biomarker to detect ED in hypertension.

According to **Armitage 1999**, Periodontitis was classified into chronic, aggressive, necrotizing ulcerative periodontitis and periodontitis as a manifestation of systemic disease. However, a new system of periodontal classification has been adopted, in which the types of disease that were previously identified as "chronic" or "aggressive" are now classified under one category ("periodontitis") and re-diagnosed based on many variants (staging and grading system)

The stage is based on the severity of the disease and the complexity of the disease management (in terms of loss of clinical attachment between teeth, radiographic bone loss, and tooth loss), complexity and distribution. Grading provides additional information about the biological features of the disease, including history-based analysis of the progression rate of

periodontitis; progression risk assessment; analysis of possible adverse effects of treatment; and risk assessment that the disease or its treatment may adversely affect the patient's normal life.

Periodontitis is described in four stages ranging from Stage I: Initial periodontitis (CAL 1-2mm), Stage II: Moderate periodontitis (CAL 3-4 mm), Stage III: Severe periodontitis with potential for loss of dentition due to periodontitis (<4), and (CAL≥5mm) Stage IV: Severe periodontitis with potential for loss of dentition due to periodontitis (≥5) and (CAL≥5mm). Grading focuses on assessing risk factors like smoking, diabetes, and outcomes of scaling and root surface debridement. Grade A: Slow rate of progression (no CAL loss over 5 years), Grade B: Moderate rate of progression (CAL loss<2mm over 5 years), and Grade C: Rapid rate of progression (CAL loss ≥2 mm over 5 years).

Non-surgical periodontal therapy (NSPT) is the “gold standard” therapy for periodontal diseases. NSPT aims at mechanical removal of bacterial plaque from the tooth surface. NSPT results in reduction of the microbial load in periodontal pockets favoring the presence of gram-positive bacteria and reducing gram-negative bacteria. Also, NSPT results in reducing the levels of proinflammatory cytokines.

In a study conducted by **Kumar in 2020**, There was a significant reduction in the Endocan ($p < 0.01$) among all the groups after NSPT. This study also suggested that Endocan levels might be increased in periodontal disease and decreased after periodontal treatment.

On the other hand, there is limited evidence that NSPT could improve arterial blood pressure, but many studies appear to support a positive correlation between severe periodontitis and hypertension. Patients with periodontitis exhibited higher mean SBP and DBP when compared with non-periodontitis patients. Also, prevalence of hypertension is higher in periodontitis patients. Systemic biomarkers like CRP, TNF-α and IL (interleukin)-1β and Endocan were elevated in patients with hypertension and periodontitis. Periodontal attachment loss was higher among hypertensive patients in comparison to non-hypertensive patients.

To the best of our knowledge, no previous study correlated between clinical parameters and the levels of Endocan and TNF-α before and after NSPT in stage II periodontitis in individuals with controlled hypertension in comparison to healthy (non-hypertension) individuals.

III. Research Question

"What is the effect of NSPT on the clinical periodontal parameters and its correlation to changes in GCF levels of Endocan and TNF-α in hypertensive periodontitis patients compared to non-hypertensive patients?"

PICOTS Elements:	
Patient/Problem	Stage II periodontitis patients with controlled hypertension.
Intervention	Non-Surgical periodontal therapy.
Comparator	Stage II periodontitis healthy individuals.
Outcome	-Clinical evaluation as a primary objective. -Biochemical evaluation as secondary objective. -Blood pressure measurement as secondary objective.
Time	-Baseline and 3 months (for clinical evaluation) -3 months (for biochemical analysis) -3 months (for blood pressure evaluation)
Setting	Faculty of Dentistry, Ain Shams University.

IV. Aim of the Study:

1- To correlate changes in GCF level of Endocan and TNF-α with changes in periodontal parameters after NSPT in both groups with clinical parameters.

2- The aim of the present study is to investigate changes in the levels of Endocan and TNF-α in stage II periodontitis patients with controlled hypertension in comparison to healthy subjects before and after NSPT.

3- To correlate blood pressure changes before and after NSPT.

Primary outcome:

Clinical evaluation of periodontal parameters plaque Index, gingival index, probing depth and clinical attachment level comparing between hypertensive and non-hypertensive groups.

Secondary outcome (s):

- Biochemical evaluation of GCF levels of Endocan and TNF-α in hypertensive and healthy subjects by ELISA.
- Systolic and Diastolic Blood pressure measurements before and after NSPT in hypertensive vs healthy individuals and check if NSPT reduces SBP and DBP.

Clinical Relevance:

To understand the role of NSPT in stabilizing the periodontal condition as well as reduce GCF levels of Endocan and TNF-α which will subsequently result in better hypertension control and improve the lifestyle.

V. Hypothesis:

Hypertension influences response to NSPT and GCF levels of Endocan and TNF-α.

VI. Ethical considerations

•**Risk and discomfort of patients**: This research will be conducted with considerations of patients' safety and with attempts to reduce patients' discomfort by reassurance.

•**Minimization of the risk:** Patients' baseline vitals will be taken before any clinical intervention to ensure patients' safety. Moreover, all medical operations will be conducted under standardized infection control

procedures.

•**Criteria for Discontinuation of Study/patient:** Patients will be allowed to leave the study if he/ she didn't confer with the oral hygiene instructions, this will be ensured by 2 weeks, 4 weeks and 6 weeks follow-up after NSPT and assessing clinical parameters like Plaque index (PI) to determine how compliant is the patient with oral hygiene instructions. Patients with PI 2 will be eliminated. Patients with uncontrolled hypertension (SBP greater than or equal to 140mmHg and DBP greater than or equal to 90mmHg) will be eliminated.

•**Benefits to the Patients and to the Community**: Patients will receive NSPT and oral hygiene instructions with no cost. The community will benefit from this study by having a better diagnostic modality for moderate cases of periodontitis and preservation of dentition. NSPT is expected to improve patients' oral/overall health and quality of life and to be of relevance in the management of patients with hypertension.

•**Privacy:** All patients' identification including patient data and photographs will be locked and not shared.

•**Confidentiality:** Patient's data will be treated with utmost confidentiality. No personnel other than the researchers will have access to the patients' data.

•**Data Management:** All patients' data will be saved on researcher's personal laptop that are not connected to any internet connections and no copies will be made in any kind of database.

•**Consent Procedures if Applicable**: Any medical procedures and photographs will have a printed written consent form.

•**Patient Informed Consent Form:** A proper consent form will be formulated &signed by the patient before starting any procedure.

VII. Study Design:

Case-control study.

VIII. Materials and Methods:

- **Study Setting:** Faculty of Dentistry, Ain Shams University.
- **Sample Size Calculation:**

A power analysis was designed to have adequate power to apply a two-sided statistical test of the null hypothesis that there is no difference would be found between tested groups regarding clinical evaluation. By adopting an alpha (α) level of (0.05), a beta (β) of (0.2) (i.e., power=80%) and an effect size (d) of (1.53) calculated based on the results of a previous study; the total required sample size (n) was found to be (26) cases. Total sample size was increased by (25%) to account for possible dropouts during follow-up intervals to be (34) cases (i.e., 17 cases per group). Sample size calculation was performed using G*Power version 3.1.9.7. Increase to 20 cases per group to compensate for dropouts. (28)

- **Eligibility criteria:**

Inclusion Criteria

1. Patients with stage II periodontitis and controlled hypertension (self-reported history of a diagnosis of HTN by a physician and blood pressure (SBP < 140 and DBP < 90).
2. Both genders aged from 30-70.
3. Minimum 20 natural teeth excluding third molars.
4. Good compliance with the plaque control instructions following initial therapy.
5. Availability for follow-up and maintenance program.

Exclusion criteria:

1. Smokers.
2. Pregnant and lactating females.

3. Patients with other systemic diseases, such as diabetes mellitus, rheumatoid arthritis and cancer.

(According to Cornell Medical Index-Health Questionnaire).

4. Patients taking antibiotic, anti-inflammatory, and immunosuppressive therapy during the preceding 3 months before the start of the trial and during the study.
5. Patients who have undergone any periodontal therapy in the last 6 months.

Justification for Exclusions:

To reduce any confounding factors and bias that may affect the results of this study.

Study procedures:

1- **Details of the interventions, testing and follow up:**

Group Case (I):

This group will include 20 patients diagnosed with stage II periodontitis and controlled hypertension. They will receive NSPT and oral hygiene measures will be instructed following treatment.

Group Control (II):

This group will include 20 patients diagnosed with stage II periodontitis and no hypertension. They will receive NSPT and oral hygiene measures will be instructed following treatment.

Study protocol and surgical steps:

1. Before enrollment, a detailed case history will be recorded.
2. A calibrated standard aneroid sphygmomanometer. The average of 3 blood pressure values (systolic blood pressure SBP and diastolic blood pressure DBP), will be taken at 1 minute interval, this will be used in data analyses.

(2020 International Society of Hypertension Global Hypertension Practice Guidelines)

3. For all patients who are suitable for the study the following clinical evaluation parameters will be measured:
   a) Plaque index (PI) (Silness&Löe, 1964)
   b) Gingival index (GI) (Löe&Silness, 1963)
   c) Probing depth (PD) (Caton, 1980).
   d) Bleeding on probing (BOP) (Ainamo, 1985)
   e) Clinical attachment level (CAL) (Ramfjord, 1967).
   f) Standardized periapical radiograph. (Pananou, 2017)

Note: Full mouth assessment will be done and then the deepest site will be evaluated for the GCF sample.

4. Baseline GCF samples will be taken the day after patients were clinically evaluated to prevent contamination with blood related to the probing of inflamed areas.
5. The sample areas will be insulated with cotton rolls to prevent saliva contamination and all supragingival plaque will be eliminated. The paper strips will be placed into the periodontal pocket and then permitted to remain for 30 seconds.
6. All patients will undergo full mouth NSPT, this will be done using ultrasonic and hand instruments.
7. The patients will receive oral hygiene instructions including tooth brushing using modified bass technique. All patients will be provided with toothbrush (soft), toothpaste (signal) and interdental cleaning with dental floss or interdental brush.
8. Two weeks follow-up to ensure that appropriate oral hygiene instructions are followed. The clinical parameter to be assessed will be PI and BOP, patients with PI 2 and BOP > 10% will be eliminated from the study.
9. Then clinical evaluation, collection of samples and measuring of SBP and DBP will be performed at 3 months after NSPT in both groups.

Outcomes Assessment:

- Clinical assessment.
- Biochemical assessment.
- Blood pressure measurement.

For clinical assessment the following clinical parameters including PI, GI, PD, BOP and CAL will be recorded for each individual at 1 site at baseline and 3 months postoperatively. Clinical parameters of PI and BOP will be assessed at 2 weeks, 4 weeks and 6 weeks follow ups to ensure proper oral hygiene. Regarding biochemical analysis, GCF samples will be collected preoperative and 3 months after NSPT. Blood pressure measurements will be taken before and after 3 months of NSPT.

a) Clinical Assessment

The following clinical parameters will be taken using UNC15 Probe at baseline and 3 months postoperative.

- **Plaque index: - (*Loe and Silness ,1963*)**

  0 = no plaque.

  1 = plaque recognized only by running a probe across the tooth surface.

  2 = plaque visible to the naked eye.

  3 = abundance of soft matter.

- **Gingival index: -(*Loe and Silness ,1963*)**

  0 = Normal gingiva

  1 = Mild inflammation – slight edema. No bleeding on probing

  2 = Moderate inflammation -redness, edema and glazing. Bleeding on probing.

  3 = Severe inflammation – marked redness and edema. Ulceration. Tendency for spontaneous bleeding

- **Probing depth :*(Ramfjord, 1959)***

Will be measured from the gingival margin to the depth of the pocket at four points (mesio-facial, mid-facial, disto-facial and mid-lingual) to the nearest millimeter using UNC-15 periodontal probe The average of the three facial points will be recorded as the facial probing depth (FPD), while the mid-lingual point will be recorded as the lingual probing depth.

- **Clinical attachment level (CAL)**:***(Ramfjord et al., 1975).*** Will be measured from the CEJ to the depth of the periodontal pocket.

b) <u>Biochemical Assessment</u>:

Biochemical assessment will be done at the deepest pocket at baseline, and 3 months after non-surgical periodontal therapy. Endocan and TNF-α levels will be determined using commercial human Endocan (ESM-1) and TNF-α ELISA Kits. Measurements will be performed according to the manufacturer's instructions.

<u>c) Blood Pressure measurement:</u>

Blood pressure measurement will be done according to, 2020 International Society of Hypertension Global Hypertension Practice Guidelines.

IX. Statistical Analysis

The obtained results will be collected, tabulated, and subjected to appropriate statistical analysis.

X. Funding of the Study

This study will be personally funded by the researcher.

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